PATENT COOPERATION TREATY

PCT

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB2005/000761	24.03.2005	26.03.2004

International Patent Classification (IPC) or both national classification and IPC
C12Q1/68

Applicant
QIAGEN AS

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IB2005/000761

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IB2005/000761

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-19
	No:	Claims	20-23
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-23
Industrial applicability (IA)	Yes:	Claims	1-23
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V.

1 Reference is made to the following documents:

D1: WO 2004/001015 A (PEL-FREEZ CLINICAL SYSTEMS, LLC; WANG, LU; XIANGJUN, LIU) 31 December 2003 (2003-12-31)

D2: WO 02/20837 A (PYROSEQUENCING AB; THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR) 14 March 2002 (2002-03-14)

2 **NOVELTY** (Art. 33(2) PCT)

2.1 D1 discloses a kit (p. 33, par. 2) that is suitable for determining one or more nucleic acid sequences that comprises one or more primers complementary to a region of common sequence, four different dideoxy nucleotides as terminator nucleotides and implicitly the enzymes that are necessary to perform a pyrosequencing reaction (example 3, p. 18). D1 thus discloses all the technical features of claims 20-23 in combination.

2.2 D2 discloses a kit that is suitable for determining one or more nucleic acid sequences by disclosing one or more primers complementary to a region of common sequence (example 3), dideoxy nucleotides that are used as terminator nucleotides (p. 19) and the enzymes that are necessary to perform a pyrosequencing reaction (p. 24) including a nucleotide degrading enzyme (p.22 l. 1-15). D2 thus discloses all the technical features of claims 20-23 in combination.

2.3 In the light of D1 and D2 claims 20 - 23 are not novel in the sense of Art. 33(2) PCT.

3 **INVENTIVE STEP** (Art. 33(3) PCT)

3.1 Regarding the subject matter of claim 1 D1 is regarded as closest prior art: D1 discloses a method for determining a target nucleic acid sequence, wherein the target nucleic acid sequence is comprised in a preparation comprising a non-target nucleic acid sequence. The method can be used to detect the presence of a plurality of alleles in a sample with mixed templates (p. 30 par. 3, example 3). One allele can be

regarded as target, the second allele as non-target. Said target nucleic acid sequence in D1 and the non-target nucleic acid sequence each are having a first region of common sequence upstream of a first region of dissimilar sequence, (p. 37). The method of D1 comprises: (a) contacting the preparation with an oligonucleotide primer complementary to at least a portion of the first region of common sequence, under conditions to hybridise the primer thereto; and (b) subjecting the preparation to a sequencing reaction, and the method further comprises a step of blocking the sequencing reaction between the primer and the non-target nucleic acid sequence. The extension of single sequencing primers is blocked by the addition of a specific dideoxynucleotides (p. 38). The blocking of a primer depends on the nucleotide of the target sequence at the 3' end of the primer sequence. Primers that are not blocked are further extended in order to determine the respective target sequence (example 3). D1 discloses as well the determination of a plurality of target nucleic acid sequences (SNPs, see example 3) in one preparation.

3.2 The difference of D1 to the subject matter of claim 1 is that the target nucleic acid in claim 1 comprises a second region of dissimilar sequence and that the sequencing reaction proceeds into the second region of dissimilar sequence of the target nucleic acid sequence, thereby determining at least the second region of dissimilar sequence of the target nucleic acid sequence.

3.3 The technical effect of the difference is that the sequence of the second region of dissimilar sequence of the target nucleic acid sequence is determined without the production of sequencing product of the non-target sequence that comprises a common sequence upstream and a first region of dissimilar sequence compared to the target sequence..

3.4 Consequently the problem to be solved by the underlying application is to provide a method to determine the sequence of a second region of dissimilar sequence of the target nucleic acid sequence without the production of sequencing product of the non-target sequence that comprises a common sequence upstream and a first region of dissimilar sequence compared to the target sequence.

3.5 The solution provided by the underlying application is a method in which the sequencing reaction of the non-target nucleic acid is blocked at the first region of dissimilar sequence and the sequencing reaction of target nucleic proceeds into the second region of dissimilar sequence.

3.6 This solution cannot however be regarded as involving an inventive step for the following reasons: In the method of D1 the sequencing reaction of a primer is stopped for the non-target , as well as for the target nucleic acid at the first region of dissimilar sequence. In the same sequencing assay primers for other target molecules (further SNPs) are extended in order to determine the sequence of said molecules (example 3). Confronted with the problem of having to provide a method to determine the sequence of a second region of dissimilar sequence of the target nucleic acid sequence without the production of sequencing product of the non-target sequence, the person skilled in the art would modify the method of D1 and would , having blocked the sequencing reaction of the non-target nucleic sequence (example 3), proceed the sequencing reaction of the target sequence into the second region of dissimilar sequence in order to determine its sequence. The person skilled in the art would do this without an inventive step. Said modification represents merely one of several straightforward modification from which the skilled person would select, given the teaching of D1 without the exercise of inventive skill, in order to solve the problem posed. This is especially the case since D1 explicitly teaches this modification for the genotyping of allotypes (page 22 l. 3-8). D1 teaches to use the method of D1 to determine the sequence of one allotype by blocking the sequencing of the other allotype, representing the non-target sequence.

3.7 In the light of D1 independent claim 1 does not fulfil the requirements of inventive step of Art. 33(3) PCT.

3.8 The same reasoning applies mutatis mutandis for the independent claims 14 and 18. D1 discloses already well the determination of a plurality of target nucleic acid sequences (SNPs, see example 3) in one preparation.

3.9 In the light of D1 dependent claims 2-13,15-17 and 19 do not to contain any additional features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step. The technical features of

said claims are either already disclosed in said document or they merely relate to obvious variations or modifications that belong to the common knowledge of the person skilled in the art.

3.10 In the light of D1 claims 1-23 do not fulfil the requirements of inventive step of Art. 33(3) PCT.

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